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Macrocyclic *Edythea quitensis* rust on *Berberis hallii* in Ecuador

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Abstract—The full macrocyclic autoecious life cycle of five spore stages was confirmed for *Edythea quitensis* on *Berberis hallii* in the highlands of Ecuador. The spermogonial and aecial stages had not previously been recorded. Spore descriptions and DNA sequence analysis are included.

Key words—*Pucciniales,* phylogeny, taxonomy

Introduction

Reports of rust occurring on *Berberis* in Ecuador date from 1891, when Lagerheim collected and distributed specimens of two species that he labeled as "*Sphenospora quitensis*" and "*Diorchidium berberidis*"; subsequently, Arthur (1918) published descriptions of these specimens as *Uropyxis quitensis* and *Sphenospora berberidis.* Jackson (1931) proposed a new genus *Edythea* to accommodate three South American species of rust on *Berberis:* the two collected by Lagerheim (recombined as *E. quitensis* [the type species] and *E. berberidis* (Lagerh. ex Arthur) H.S. Jacks.) and a new species from Bolivia (*E. tenella* H.S. Jacks. & Holw.). Jackson (1931) depicts *Edythea* as having urediniospores and teliospores "borne in a very characteristic and presumably unusual manner" because of the lack of proper sori, as the mycelial threads emerge from the stomata without rupturing the leaf tissue to give rise to spores. The characteristics described by Jackson (1931) to differentiate between the three species of *Edythea* were the presence of a vertical septum on teliospores for *E. berberidis,* a variable septum with nearly globoid teliospores for *E. quitensis,* and a variable septum but with ellipsoid teliospores for *E. tenella.* Urediniospores from the three species are described as quite similar, but there is no mention of any spore stage, other than urediniospores or teliospores, for the three *Edythea* species described (Jackson 1931).

The host family of these rusts, the *Berberidaceae,* includes 13 genera, of which only the genus *Berberis* L. is present in Ecuador. With over 500 species worldwide, *Berberis* (including *Mahonia* Nutt.) is the largest genus of the family. There are two important centers of diversity for *Berberis,* one in Eurasia with around 300 species mostly in China and the Himalayas and another in South America with approximately 200 species (Ahrendt 1961). The simple leaf type occurs throughout South America, while the compound leaf type (previously referred to *Mahonia*) does not reach South America, and is found only from Mexico to Costa Rica (Ulloa 2013). In Ecuador, 32 *Berberis* species have been reported, of which 16 are classified as endemic (Ulloa 2013). All species occur in the highlands over 2000 meters above sea level. The *Berberis* plant is used locally for coal, fencing, and firewood, with the fruit used in jams and the roots used for medicinal purposes and staining (Ulloa 2013).

In this paper we report the rust fungus *Edythea quitensis* found on *Berberis hallii* Hieron. in Ecuador, as having a full macrocyclic autoecious life cycle; sequence analysis of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA for three spore types also illustrates the phylogenetic relationship between the *Edythea* rust samples collected on *B. hallii* in Ecuador with rust collected from other *Berberis* species elsewhere.

Materials & methods

Collection and spore morphology

Rust infected leaves were collected from *Berberis hallii* in Quito, Pichincha province. Fresh spores were examined microscopically in water, and teliospores, urediniospores, and aeciospores were measured using Infinity Analysis software (Lumenera Corp., ON, Canada). Twenty random spores from each collection were measured for each spore type. Germinating teliospores, basidiospores, and spermogonia were also observed. Collections were deposited in the QCAM fungarium at Pontifica Universidad Católica del Ecuador, Quito, Ecuador (Table 1).

Molecular analysis

DNA was extracted separately from aecia/spermogonia and uredinia/telia found on different collections of *B. hallii* leaves. The tissue was macerated with zirconium

Table 1. *Edythea quitensis* samples analyzed

Specimen	Geographical coordinates	Altitude [m a.s.l.]	Spore type observed*	GenBank no.
QCAM6449	−0.18646, −78.46178	2890	II, III	KX298148
QCAM6450	−0.17609, −78.46702	2917	0, I, II, III, IV	KX298149
QCAM6451	−0.17455, −78.46968	2879	II, III	KX298150
QCAM6452	−0.17711, −78.46658	2919	II, III	KX298151
QCAM6453	−0.17497, −78.46890	2890	II, III	KX298152
QCAM6454	−0.17708, −78.46670	2887	0, I, II, III, IV	KX298153

* Following Cummins & Hiratsuka 1983

Table 2. Rust sequences from GenBank included in the phylogenetic analysis

GenBank no.	Taxon	Country	Host	Reference
JX047488	*Puccinia* sp.	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047486	*Puccinia* sp.	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047489	*Puccinia* sp.	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047484	*Puccinia* sp.	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047456	*P. graminis* f.sp. *tritici*	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047465	*P. graminis* f.sp. *tritici*	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047468	*P. graminis* f.sp. *tritici*	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047467	*P. graminis* f.sp. *tritici*	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047477	*P. graminis* f.sp. *avenae*	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047478	*P. graminis* f.sp. *avenae*	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047482	*P. graminis* f.sp. *avenae*	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047475	*P. graminis* f.sp. *avenae*	Sweden	*Berberis* sp.	Berlin et al. 2013
AY956558*	*P. striiformoides*	Iran	*Dactylis glomerata*	Abbasi et al. 2004
DQ417406*	*P. striiformis* f.sp. *tritici*	USA	*Triticum aestivum*	Barnes & Szabo 2007
GU382672	*P. striiformis* f.sp. *tritici*	USA	*Berberis chinensis*	Jin et al. 2010
GQ457304+	*P. pseudostriiformis*	USA	*Berberis* sp.	Jin et al. 2010
GQ457306+	*P. pseudostriiformis*	USA	*Berberis koreana*	Jin et al. 2010

* Best Blastn hits (from non-*Berberis* hosts) to *Edythea quitensis* ITS sequence
+ Formerly *Puccinia striiformis* f.sp. *poae* (Jin et al. 2010)

beads and soaked in 0.5% SDS lysis buffer at 55°C for 3 hours. Protein precipitation was done with 4M guanidine isothyocianate solution, pH 7.5, followed by isopropanol and ethanol precipitation. The ITS (ITS1+5.8S+ITS2) region was amplified with primers ITS1F (Gardes & Bruns 1993) and ITS4BR (Vialle et al. 2009). The PCR cocktail comprised 0.2 μM of each primer, 1X PCR buffer, 3mM $MgCl_2$, 0.2mM dNTPs, 0.1 U/μL Platinum Taq DNA polymerase (Invitrogen, Brazil), and 1ng/μL of DNA. Amplification conditions were: 94°C for 3 min., 30 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 60 s, and a final extension at 72°C for 10 min. PCR products were sequenced at Macrogen Inc. (Seoul, South Korea). Sequences were edited in Geneious R9 (Kearse et al. 2012) and submitted to GenBank (http://www.ncbi.nlm.nih.gov). The alignments were deposited into TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S19447).

Sequence data were analyzed using Maximum Likelihood with a Custom (010212) Substitution model determined by jModelTest 2.0.2 (Darriba et al. 2012, Guindon & Gascuel 2003) according to Corrected Akaike Information Criterion (AICc). Sequence data available on GenBank from rust fungi infecting *Berberis* species from other countries were included in the analysis, as well as the two best Blastn hits (Table 2). A bootstrap of 1000 replicas was used.

Taxonomy

Edythea quitensis (Lagerh. ex Arthur) H.S. Jacks. & Holw.,
Mycologia 23: 99. 1931. Fig. 1

≡ *Uropyxis quitensis* Lagerh. ex Arthur, Bot. Gaz. 65: 464. 1918.

Type: Ecuador, Quito, stages II and III on *Berberis* sp., April 1891, G. Lagerheim (PUR 68850, holotype, originally deposited as "*Sphenospora quitensis*").

Spermogonia, aeciospores, urediniospores, teliospores, and basidiospores were observed on *B. hallii* in Quito. A total of six rust samples were analyzed, two showing all five spore stages and four only urediniospores and teliospores (Table 1). Spermogonia and aecia were found occurring simultaneously on leaves several times during the year, usually after rains.

Spermogonia were observed on the upper side of the barberry leaves, and aecial cups were present on the underside of the leaves. Spermogonia were yellow initially, becoming more orange in color as they aged. Aecial cups were bright orange. Aeciospores were ellipsoid to globose, 27.3–21.5 × 27.0–20.9 µm, verrucose, with a thin wall, yellow-orange in color. Urediniospores and teliospores were found year round and matched characteristics described by Jackson (1931); specifically: both spore types emerged through the stomates without rupturing the tissue and were cinnamon-brown in color; urediniospores were mostly globoid, single-celled, 29.5–22.8 × 28.2–22.6 µm; teliospores were globoid, two-celled, 27.6–22.0 × 31.6–24.0 µm with pedicels 21.4–30.0 × 5.5–6.6 µm. Germinating teliospores were observed forming a basidium with four basidiospores on sterigmata.

Phylogeny

ITS sequence analyses obtained separately for aecia/spermogonia and for uredinia/telia supported all within the same species (Fig. 2). The Ecuadorian samples were distinct from several rust species reported from *Berberis*

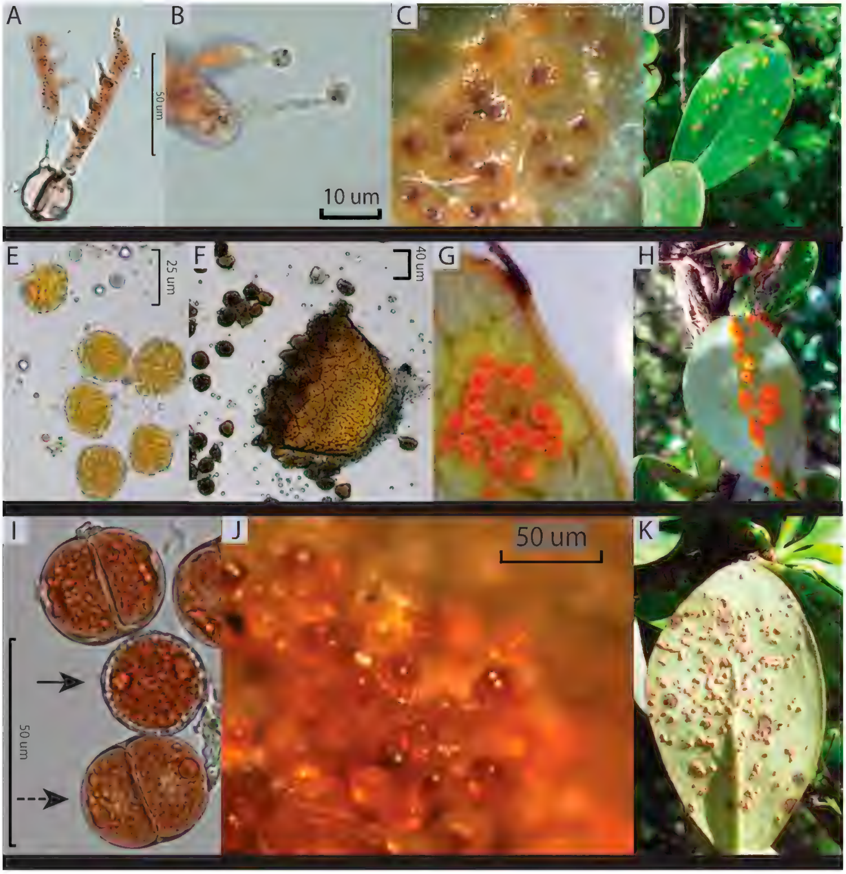

Fig. 1. *Edythea quitensis:* macrocyclic stages. A. basidia; B. basidiospores on sterigmata; C. spermogonia; D. spermogonial infection on upperside of barberry leaf; E. aeciospores; F. aecial cup; G. closeup of aecial cups on underside of barberry leaf; H. aecial infection on underside of barberry leaf; I. teliospore (dashed arrow) and urediniospore (whole arrow); J. mix of urediniospores and teliospores; K. urediniospore and teliospore infections on underside of barberry leaf.

from Sweden and the USA. The resulting topology was well supported by bootstrap values.

Discussion

The complete macrocyclic autoecious life cycle for *Edythea quitensis* occurring on *B. hallii* is described here for the first time. The three original

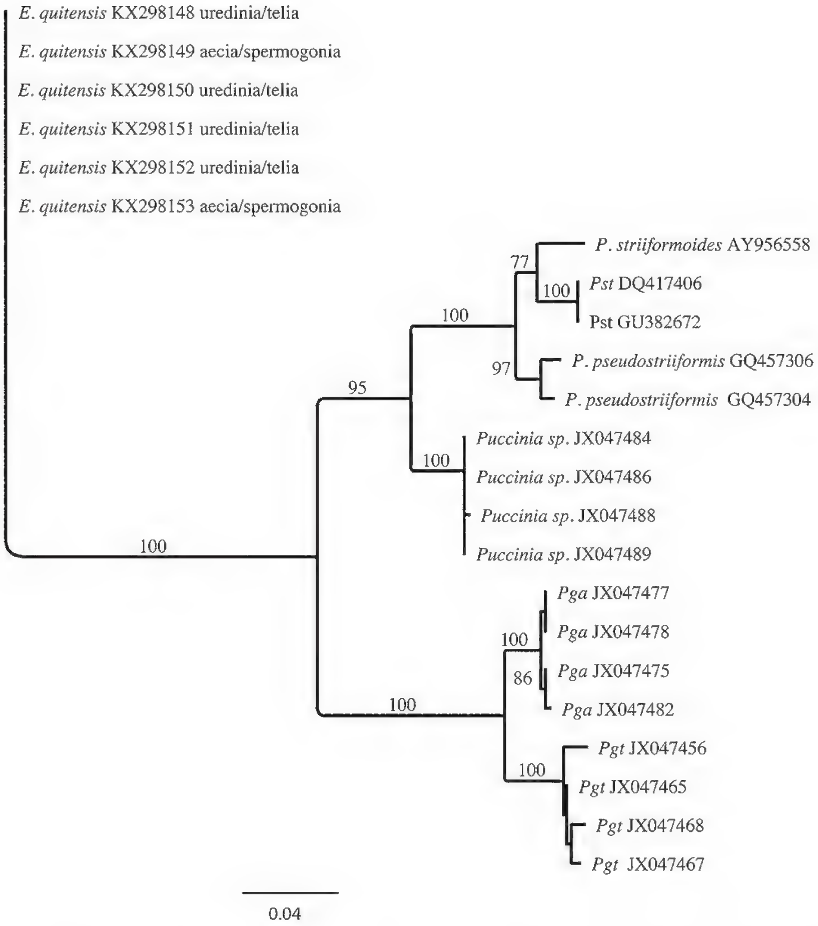
E. quitensis KX298148 uredinia/telia
E. quitensis KX298149 aecia/spermogonia
E. quitensis KX298150 uredinia/telia
E. quitensis KX298151 uredinia/telia
E. quitensis KX298152 uredinia/telia
E. quitensis KX298153 aecia/spermogonia
P. striiformoides AY956558
77
100
Pst DQ417406
Pst GU382672
100
97
P. pseudostriiformis GQ457306
P. pseudostriiformis GQ457304
95
Puccinia sp. JX047484
100
Puccinia sp. JX047486
Puccinia sp. JX047488
100
Puccinia sp. JX047489
Pga JX047477
Pga JX047478
100
86
Pga JX047475
100
Pga JX047482
Pgt JX047456
100
Pgt JX047465
Pgt JX047468
Pgt JX047467
0.04

Fig. 2. Maximum Likelihood phylogenetic tree based on the ribosomal DNA sequence of the ITS1+5.8S+ITS2 region of *Edythea quitensis* and representative taxa of rust from *Berberis* spp., plus the two best Blastn hits not found on *Berberis* spp. One thousand bootstrap replicates were used; values ≥70% are given above branches.

Taxonomic abbreviations: *Pst* = *Puccinia striiformis* f.sp. *tritici; Pga* = *P. graminis* f.sp. *avenae; Pgt* = *P. graminis* f. sp. *tritici.*

Edythea rusts on *Berberis* and a fourth species, *E. soratensis* Ritschel (described from a 1920 collection on *Berberis* in Bolivia; Berndt et al. 2007), have not previously been subjected to repeated seasonal field observations. However, the increased attention being paid to *Berberis* spp. as potential alternate hosts for stripe rust *Puccinia striiformis* Westend. in wheat growing areas (Jin et al. 2010, Zhao et al. 2013) afforded the opportunity for closer examination of the rust species occurring on *B. hallii* in Quito. It is likely that the original description of *E. quitensis* did not include the spermogonial and aecial stages due to the restricted time and number of collections that were available at that time.

The use of ITS sequence comparison of the different spore stages confirmed that the aeciospores, urediniospores, and teliospores analyzed belong to the same species.

Ecuador is within the center of diversity of *Berberis* species in South America, and more work is needed to describe the rust fungi that infect these hosts. Rust species description and identification are important for managing wheat rust resistance in Ecuador and adjacent regions as well as for documenting fungal biodiversity in these countries.

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